Picosecond fluorescence of reaction centres from
*Rhodospirillum rubrum*


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The fluorescence kinetics of isolated *Rhodospirillum rubrum* reaction centres was studied. In the picosecond time range the decay was well approximated by two exponential components. The short-lived component was observed only when P*700 was reduced and had a maximal amplitude at about 910 nm. Its lifetime was equal to 7 ± 2 ps both at room temperature and at 77 K. It is inferred that this fluorescence component is emitted by excited photochemically active P*700 before the formation of an intermediate radical-pair state. The second, long-lived component with a lifetime of about 100–200 ps was observed in a wider spectral range and had maxima at about 850 and 900 nm both under reducing and oxidizing conditions. The bulk of this luminescence seemed to be emitted either by photochemically inactive P*700 and/or by traces of antenna bacteriochlorophyll.

1. INTRODUCTION

Picosecond absorption studies of primary charge separation at the reaction centres (RCs) of photosynthetic bacteria showed that the formation of an intermediate ion-radical pair state [P*700^{-}I] (where P*700 is the oxidized special-pair bacteriochlorophyll and I is reduced bacteriopheophytin (BPh) or a complex of BPh and P*800) proceeds in 4–9 ps [1–4]. After 150–250 ps the electron from I moves to the primary acceptor of a non-porphyrine nature, Q [1,5]. If Q is extracted or reduced chemically, charge separation still proceeds in less than 10 ps [5], but the lifetime of the radical-pair state [P*700^{-}I] is lengthened to about 10 ns [5]. Since primary charge separation is initiated from the singlet excited state of P*700, the picosecond kinetics of fluorescence decay of isolated RCs enables an independent approach to the investigation of the kinetics and energetics of the intermediate steps of excitation energy stabilization on RCs. So far picosecond fluorescence of bacterial RCs has been studied only in Rubin's laboratory [6–8], where a picosecond emission component with the lifetime 15 ± 8 ps was discovered. However, this value exceeds that of the primary charge separation determined by picosecond absorption studies and, besides, it was reported to lengthen to 60 ps when the temperature was lowered to 77 K [8]. The latter observation is inconsistent with the known temperature independence of the quantum yield of primary charge separation, which remains close to unity in the whole temperature range from 300 to 5 K [9]. Moreover, picosecond absorption measurements with *Rhodopseudomonas sphaeroides* R-26 PCs showed that the rate of electron transfer from I to Q is even higher at low temperatures [10].

The present work describes the results of picosecond fluorescence measurements with RCs from *Rhodospirillum rubrum*. Even for the purest RC preparation fluorescence was found to be composed of two components: the short-lived component with a lifetime of 7 ± 2 ps (independent of
temperature) and the long-lived component with a lifetime of 100–200 ps. The short-lived emission was observed only when P\textsubscript{870} was capable of photooxidation, while the long-lived one was detected both at oxidizing and reducing conditions.

2. MATERIALS AND METHODS

RCs were isolated from \textit{R. rubrum}, wild-type MGU, by a modified method of Noël and Gingras [11]. Low redox potentials were obtained by adding solid Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} (approx. 1 mg/ml). For low temperature measurements, RC suspensions in 0.05 M Na-P buffer, pH 8.0, 0.01% LDAO, were diluted 3-fold with glycerol.

Picosecond fluorescence measurements were performed as described [12]. Fluorescence was excited by a ‘Spectra-Physics’ mode-locked CW oxazine 750 dye laser (pulse duration 3 ps) synchronously pumped with 82 MHz by a krypton-ion laser and registered through a monochromator by a synchroscan streak camera. The instrumental time resolution was 3 ps and the spectral one 1 nm.

3. RESULTS AND DISCUSSION

Absorption and fluorescence spectra of \textit{R. rubrum} RC preparations at room temperature are shown in fig.1. It appears convincing that the optical characteristics of these preparations correspond to those of the purest ones described in literature: \(A_{280}/A_{800} = 1.15–1.25\) and \(A_{764}/A_{802}/A_{865} = 1.0/2.3/1.0\). The fluorescence maximum of reduced RCs is shifted about 10 nm to the red with respect to that of intact chromatophores. This means that the Stokes shift is twice as large for P\textsubscript{870} as for light-harvesting bacteriochlorophyll. Besides, the fluorescence spectrum of the RCs is markedly broader. Fig.2A shows the fluorescence decay kinetics for RCs at 910 nm after excitation by 800 nm picosecond light pulses. The decay is evidently nonexponential and is well approximated by two components: the short-lived component with the lifetime \(\tau_1 = 6 \pm 1\) ps and the long-lived component with \(\tau_2 = 90 \pm 10\) ps, the relative amplitudes being \(A_1:A_2 = 3.5:1\). Measurements with different preparations, employing excitation at 385 nm (Soret band), 760 nm (RC BPh) and 800 nm (P\textsubscript{800}) with varying excitation density from about \(10^8\) to \(10^{11}\) photons/cm\textsuperscript{2} per pulse in the presence and in the absence of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}, showed that the spread of \(\tau_1\) values is within 7 ± 2 ps. The lifetime of the long-lived component \(\tau_2\) varied under these conditions from 90 to 240 ps. The short-lived component was observed only at \(\lambda > 820\) nm with a maximum at about 910 nm, and its spectrum did not depend noticeably on excitation wavelength. The value of \(\tau_1\) was constant within experimental error all over the measured 820–970 nm spectral range. As for the long-lived fluorescence component, its spectrum and the spectral dependence of its lifetime changed with the sample preparation (and/or with the excitation wavelength). Thus, for one definite sample at 760 nm excitation this component was detected in the spectral range from 770 to 970 nm and had maxima at about 790, 850 and 900 nm. These features as well as the spectral dependence of \(\tau_2\) can be seen from the spectrochronogram of the fluorescence emission of reduced RCs, shown
in fig. 3. It follows that under these conditions the long-lived emission is heterogeneous. When (for another sample) $P_{800}$ of the RC was excited directly, $r_2$ was independent of the detection wavelength and the spectrum of the long-lived component had either one (at 900 nm) or two (at 850 and 900 nm) maxima, depending on the RC concentration and the presence of $Na_2S_2O_4$ in the sample.

While the lifetime of the short-lived component does not depend on the presence of $Na_2S_2O_4$ in the incubation medium, its amplitude increases 3-4-timcs. This increase must be caused by a transition of the RCs, photooxidized by excitation light (RC state $P^*Q^-$), into the state $PQ^-$ with $Q$ being chemically reduced. The lifetime of the long-lived component shortened under these conditions, e.g. for the sample whose absorption spectra and fluorescence decay curves are shown in figs 1 and 2, it became equal to 90 ps. Besides, a nanosecond component with the lifetime $r_3 > 2$ ns appeared. In

the absence of the agents reducing $P_{870}$ (or when the sample was specially exposed to a prolonged action of oxygen), the short-lived component was not detectable but it could be completely restored by $10^{-3}$ M additions of sodium ascorbate or mercaptoethanol. The amplitude of this component was found to be roughly proportional to the concentration of photochemically active RCs.

When the temperature was reduced to 77 K, the amplitude of the short-lived component decreased, but the lifetime remained equal to 6 ± 1 ps, i.e. like at room temperature (fig. 2B), while $r_2$ dropped down to 70 ps. The decrease in the amplitude of the short-lived component is most probably due to photoaccumulation of the RCs with $P_{70}$ in the triplet state $P^R$, since this process reduces the portion of photochemically active RCs.

The data obtained show uniquely that it is the photochemically active $P_{870}$ that is the source of the picosecond emission component, and that this fluorescence is emitted in a time interval between light absorption and the formation of the intermediate radical-pair state $[P_{870}^-]$. An excellent coincidence between our fluorescence and recent picosecond absorption data is noteworthy. The second important fact is that the primary charge
separation time was directly measured to be the same at room temperature and 77 K (see also [13]), which is in full correlation with the temperature independence of the P870 photooxidation quantum yield [9].

The 100–200 ps emission component is of a heterogeneous nature and is observed even in the purest preparations. It was detected also in [6–8]. At the 800 nm excitation the spectrum of this component resembled that of the short-lived one. Taking into account that this long-lived component is observed (though with diminished amplitude) also under the conditions when all photochemically active P870 are photooxidized, it may be concluded that it belongs either to photochemically inactive P870 or to the traces of antenna bacteriochlorophyll or both. The recombination luminescence contribution due to reversed electron transfer from the level of the intermediate state to that of the P870 singlet excited state should be low compared with the observed signal as shown by model calculations. As for the 790 nm band in fig.3, it is most probably due to BPh emission.

REFERENCES